by small circular regions are allowed to grow in size or shrink in response to forces acting on them. The growth and shrinkage of the complexes is modeled using the approach proposed by Besser and Safran (2006) where the mechanical forces from intracellular contraction, cell elasticity, and adhesion size are coupled by force induced conformational changes of molecular sized mechanosensors located in the focal adhesions. Further the cytoskeletal reorganizations driving the intracellular deformations are captured by an empirical active deformation tensor.

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A Mechanochemistry Model of Focal Adhesion Dynamics in Cell Migration

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Focal adhesions are essential to mediate cell extracellular matrix (ECM) adhesion and force transmission during cell motilities, which involve the crosstalk between physical signals such as contractile forces or membrane dynamics, and chemical signaling events such as focal adhesion kinase related regulation pathways. However, the underline mechanism of the biophysical regulations of force transmission among actin cytoskeleton, cell membrane, focal complex and ECM remains poorly understood. We constructed a mathematical model to understand the behavior of focal adhesion complex under different experimental conditions. By integrating the cell membrane dynamics, actin network fluid dynamics, and the mechanochemistry of focal complex, the model reveals itself the capability to capture the essential characteristics of focal adhesions in cell motility. In particular, the model explains the focal adhesion growth pattern at different ECM stiffness. The model thus provides a comprehensive vision of the focal adhesion dynamics.

Unconventional Myosins

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Class-1 Myosins at the Interphase between Spindles and Membranes

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Class-1 myosins play key roles in the dynamic organization of the cytoskeleton and membrane shape as they act as divalent cross-linkers interconnecting and generating force between both actin and membrane systems. Previously we have shown that the long-tailed Dictyostelium myosin-1C not only is involved in membrane remodeling during endocytic processes, but also plays a role in spindle dynamics during mitosis through direct microtubule interactions. Here, we characterized myosin-1D as the second myosin-1 member with roles in mitosis. Our studies reveal that myosin-1D binds spindle pole microtubules and associates with the nuclear envelope of dividing cells. Myosin-1D targets microtubules and nuclear structures early during mitosis and decorates the membrane embedded chromatin masses until the end of cell division. This suggests a role of the motor in nuclear membrane fission by mediating microtubule-membrane interactions. Since the nuclear envelope in Dictyostelium remains intact throughout mitosis, except for regions of perforations that are found near the spindle poles, myosin-1D appears to act as a dynamic linker connecting spindle pole microtubules to the nuclear membrane important to hold the envelope together as it gets stretched upon spindle elongation. To investigate the mechanisms that govern myosin-1s to distinct mitotic structures, we have generated a set of motor and tail domain constructs and characterized binding affinities and kinetics of intermolecular interactions with lipid membranes, actin, and microtubules. Our results show that differences in strength and specificity of lipid and microtubule binding and regulatory roles of the tails account for the specialized functions of the motors in mitosis.

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Asymmetric Motility Powered by Myo1c on Lipid Membranes

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Class-I myosins are single-headed motors that link cell membranes to the underlying actin cytoskeleton. Actin binding occurs via the motor domain, while membrane binding has been proposed to occur via the tail domain that, in some isoforms, has been shown to interact with phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) through a putative pleckstrin homology domain. To study the interplay between myosin-I, actin, and membranes, we reconstituted actin gliding motility on a membrane using full-length vertebrate myo1c bound to fluid (D ~ 1 μm2/s) supported lipid bilayers (SLBs) composed of 2% PtdIns(4,5)P2, 97.9% DOPC, and 0.1% TRITC-PE. In this system, myo1c dynamically attaches and detaches to/from the lipid bilayer and actin filaments. The actin-gliding velocity on SLBs at 22 °C (~ 20 nm/s) is comparable to that of myo1c rigidly anchored to a non-fluid surface via a tail-binding monoclonal antibody. Strikingly, the gliding of actin filaments on SLBs occurs along curved paths in a counterclockwise fashion (i.e., the actin filaments turn left) when viewed from the side of the membrane. This striking asymmetric motility was not observed when the myo1c was rigidly anchored to the surface via the antibody. The tail domain was not required for filament turning, as asymmetric motility was observed with a motor domain construct (no tail) attached to fluid biotinylated SLBs via a biotin-streptavidin linkage. A slight leftward bias was also observed when full-length myo1c dynamically interacts to Ins(1,4,5)P3 that is attached to the coverslip by a flexible 3-carbon linker. We conclude that class-I myosins can produce asymmetric motility on surfaces to which they can dynamically reorient. This asymmetric motility appears to be a fundamental property of the motor domain.

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Myo1c is Built for Sustained Power Under Load, not Tension Sensing

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Myo1c, a widely expressed single-headed myosin, has been proposed to act in a variety of cellular processes, including insulin-stimulated transport of GLUT4 and mechanosensing in sensory hair cells; however, the mechanical and kinetic properties of a transporter and a tension sensor are very different. To better understand the adaptations of myo1c for its molecular roles in the cell, we examined its biochemical and mechanical properties using ensemble kinetic and single molecule techniques. Consistent with previous reports, we found that the myo1c working stroke is comprised of two substeps, which are likely associated with phosphate and ADP release. Using an optical force clamp in the presence of 5 mM MgATP, we found myo1c's actin attachment lifetime to be insensitive to forces < 2 pN, and only modestly force dependent at loads > 2 pN. This 2-phase force dependence suggests that the kinetic transition that limits detachment at low loads is relatively force insensitive, and that a different, more force sensitive transition becomes rate limiting at higher forces. This tension sensing behavior is unchanged by a high dynamic loading rate in the optical trap or by a reduction in the free magnesium concentration from 1 mM to < 2 μM at 5 mM total [ATP]. The force-dependent properties of myo1c are substantially different from myo1b, a closely related myosin-I isoform, which exponentially increases its attachment lifetime over 75-fold at only 2 pN of force. The tension sensing behavior of myo1c is more consistent with its functioning as a slow, power-generating transporter rather than a tension sensor. Moreover, this result demonstrates functional diversity within the myosin I superfamily; different isoforms are mechanochemically tuned to their specific functions. Supported by NIH (GM087253) and NIH training support to MJG (AR053461 & GM097889).

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Interactions of Calmodulin and the IQ Motifs of a Type I Myosin in Fission Yeast

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Myosins are a diverse group of ATP dependant actin-associated motor proteins with varied functions ranging from muscle contraction to cytokinesis and endocytosis. In fission yeast there are three classes of myosin each interacting with a number of light chains, including two calmodulins Cam1 and Cam2. Previous work has shown that the myosin specific light chains are important for myosin function. With this in mind we have undertaken a biochemical and cell biological approach to characterise calmodulin and to investigate the specific interactions between myosins and calmodulins. Myo1, a type I myosin in fission yeast has two IQ domains and here we have used a FRET based system to characterise the biophysical interactions of calmodulin and single IQ domains of Myo1. We have determined that Cam1 only binds to IQ1, and not IQ2, with an affinity of 28 μM. Using IAANS-labelled Cam1 we establish that the pCa_{50} of Cam1 is 7.2 and calcium is released at 0.8 sec^{-1}. The affinity of Calmodulin for calcium is tighter in the absence of magnesium. In the presence of calcium there is no detectable Cam1 binding to IQ1.